

HEYDENS, WILLIAM F [AG/1000]

From: [REDACTED]@eesmail.elsevier.com on behalf of RTP (ELS) [REDACTED]@elsevier.com]
Sent: Wednesday, August 26, 2009 6:38 PM
To: HEYDENS, WILLIAM F [AG/1000]
Subject: Reviewer Notification of Editor Decision



Ref: RTP-09-41R1
Title: Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines
Article Type: Regular Article

Dear Dr. WF Heydens,

Thank you once again for reviewing the above-referenced paper. With your help the following final decision has now been reached:

Reject (allow resubmission)

The author decision letter and reviewer reports can be found below.

We appreciate your time and effort in reviewing this paper and greatly value your assistance as a reviewer for Regulatory Toxicology and Pharmacology.

If you have not yet activated or completed your 30 days of access to Scopus, you can still access Scopus via this link: http://scopees.elsevier.com/ees_login.asp?journalacronym=RTP&username=WHeydens-986 You can use your EES password to access Scopus via the URL above. You can save your 30 days access period, but access will expire 6 months after you accepted to review.

Yours sincerely,

Gio Batta Gori, DSc, MPH
Editor-in-Chief
Regulatory Toxicology and Pharmacology

To: "Gilles-Eric Seralini" [REDACTED]@unicaen.fr
From: "RTP (ELS)" [REDACTED]@elsevier.com
Subject: RTP-09-41R1: Final Decision



Ms. No.: RTP-09-41R1
Title: Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines
Corresponding Author: Prof. Gilles-Eric Seralini
Authors: Celine Gasnier; Nora Benachour; Emilie Clair; Carine Travert; Frederic Langlois; Claire Laurant; Cécile Decroix-Laporte;

Dear Prof. Seralini,

Thank you for submitting your manuscript to Regulatory Toxicology and Pharmacology. Your paper,

referenced above, has been reviewed by experts in the field. Based on their comments, I regret to inform that your manuscript - as now written - cannot be accepted for publication in Regulatory Toxicology and Pharmacology.

The reviewer comments are included below. However, the manuscript was well received, and we encourage you to resubmit a thoroughly revised manuscript for re-consideration. In this case, the revised manuscript would be considered a new submission and would be given a new manuscript number with a new date of receipt.

The critique of this paper in no way implies a lack of interest in this area of research, and I hope that you will continue to submit your work to this journal in the future.

Sincerely,

Gio Batta Gori, DSc, MPH
Editor-in-Chief
Regulatory Toxicology and Pharmacology

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Reviewers' comments:

Reviewer #3: The authors have addressed concerns raised in the first review. However, some concerns remain.

In line 289 the authors refer to clearly visible chromatin condensation in figure 6. Unfortunately, the resolution of the images provided were not good enough to allow me to see the condensed chromatin. Perhaps higher resolution images will make the authors description more obvious but for now I can't tell whether the figure conforms to the description or not. Please provide a higher resolution image.

In lines 295-296 the authors state in their description of figure 7 that "D does not enhance these cytochromes by itself". However, the figure does not show this, only that CYP induction by R is enhanced when followed by D or diminished when preceded by D. Without a D alone control we can't know what D does on its own.

In defense of the significance given by the authors to the 3A4 induction shown in figure 6, against the criticism by reviewer 4 that the induction was "very mild", I can point out that the 2-3 fold 3A4 induction that was observed is typical of HepG2, even with a strong inducer such as rifampicin (see Westerink and Schoonen, Tox. In Vitro, 2007, vol 21:1581-1591).

In line 301 the authors say "D does not modify this effect by R either before or after treatment", referring to figure 8 and the inhibition of GST activity by R. However, D does appear to "modify this effect" in that GST activity is reduced in both RD and DR treatments compared to RM.

In line 416 the authors refer to the cell lines studied as human hepatocytes. Readers that are well versed in

hepatocyte studies will object to this because the cells are in fact hepatocarcinoma cell lines that differ phenotypically from primary hepatocytes in several significant ways.

In lines 113-116 of the methods section there is an awkwardly worded sentence. What is meant by "pH 5.8 of R"?

In Line 245 should "aver" be "after"?

Reviewer #4: General Comments

Overall Conclusions: It appears that the majority of the numerous grammatical/sentence syntax problems, poor definition of abbreviations, inadequate figure legends, etc. that were present in the original manuscript have been dealt with in the revised manuscript. However, the revised manuscript is still disjointed, and it is difficult to understand what was performed; it has significant scientific flaws, and it contains speculative conclusions and inaccurately characterizes risk. In addition, the work contained in the revised manuscript remains repetitious of that contained in previous publications from this group, and as such, it is not 'new' and does not represent information that would be useful to the Journal's readers. Further, as pointed out previously, the conclusions and model presented at the end of the paper is not supported by the data developed in the paper. For example, there are large gaps in mechanistic information that are required support the explanation for modulation of cytochrome P450 expression. Therefore, it is again recommended that this revised manuscript should not be accepted for publication in Regulatory Toxicology and Pharmacology.

The authors study design makes it impossible to separate the contributions of glyphosate, the surfactant or the lack of serum to the toxicity. The experiment that must be conducted to make this paper acceptable is one that will provide independent data of all variables. Test materials must include a glyphosate-based formulation, a formulation blank (all components except glyphosate), glyphosate, the surfactant in the formulation as well as additional surfactants and these experiments must be conducted in serum and serum-free conditions. Including additional surface acting agents is critical to put the observed effects in in vitro experiments into perspective - as demonstrated by other authors even those found in personal and home care products can produce similar effects without causing unacceptable toxicity to the consumers who use them.

Furthermore, one must question the authors' objectivity given the tone and quality of their responses to comments. It appears that the authors' lack of objectivity influenced their interpretation of my original review. For example, I did not question the observed toxicity of the formulations in the in vitro studies; what I did question was the interpretation of the results based on the study design. In addition, I provided various references in my original review that had similar types of investigations for the authors to consider in reflection of their data, their interpretation of the results, and as a point of departure for their response. Yet the authors did not respond to the comments regarding these references or include them the current draft. As an example, I cited a sound, peer-reviewed study that addressed the issue of surfactant effects on in vitro systems (Levine et al., 2007), and the authors should be aware of other such work (e.g., Amouroux et al., 1998); yet the authors ignored open scientific discourse on this issue and made no mention of other references in their revised manuscript. This selective use of the literature is troublesome.

Specific Comments

Page 2, lines 40-41. It is stated that "CYP3A4 is specifically enhanced by R at doses 400 times less than used in agriculture (2%)". A similar statement appears page 13, lines 308-310 ("We tested R at sub-agricultural levels below the maximum level of residues authorized in some feed ."). These statements are apparent attempts to denote 'risk' to the liver cells of humans, but they actually reflect meaningless 'apples-and-oranges'

comparisons. In the statement made here in the Abstract, the authors compare the in vitro concentrations used in their study to what they believe is the concentration of glyphosate in spray solutions typically used in agricultural operations. On page 13, the authors compare the in vitro concentrations used in their study to the highest existing tolerance for animal feed/hay uses. Both risk assessment comparisons are scientifically invalid. If the authors wish to relate their findings at the in vitro concentrations used in their experiments to actual/anticipated human exposures, then the appropriate exposure assessment must be conducted. In any case, the two referenced statements are misleading and especially inappropriate in a journal with the stated "Aims & Scope" of Regulatory Toxicology and Pharmacology.

Dig 1 was added to these cultures at a concentration of 2%, which equates to 20,000 ppm. Observing mitigation of cytotoxicity effect after adding 20,000 ppm of an organic extract is not an unexpected result. The authors have not justified why such a high concentration of the extract was added nor have they put into context what a 2% concentration translates to in an in vivo model. The extract most likely decreases bioavailability in much the same way that addition of BSA or serum would. The authors did not make these comparisons or discuss how serum free conditions do not represent physiological conditions.

Figure 2. After closely reviewing the data, I have concluded that this is not new information but rather was taken directly from a recent publication from this group (Gasnier et al. 2009, Figure 1) and is also repetitive of their data and figure from Benachour et al, 2009 (Figure 1, same data repeated in other cell lines). The authors' response to Reviewer 3's request for clarity in Figure 2 from the original submission was to insert another graph, which is almost a "carbon copy" from Benachour et al., 2009 (figure 2). The Editor should investigate this issue and determine the authors' adherence to the Journal's "Guide for Authors", which requires a "Submission Declaration" denouncing concurrent submissions of the same data for publication in different journals. The two above-mentioned publications by this group are:

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE. 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*. 21;262(3):184-91.
Benachour N, Séralini GE. 2009. Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem. Res. Toxicol.*, 22; 97-105.

Figure 5. The effect on caspase activity after 48 h of exposure to the formulation is uninterrupted. No where in the paper do the authors show the level of cytotoxicity for the R450 formulation after 48 hours of exposure. The caspase 3/7 activity noted in this figure reflects nothing more than a measure of cytotoxicity after exposure to a supra-physiological concentration of 60 ppm R450. Additionally, the authors do not report the level of caspase activity in the control treatment, which makes it impossible to interpret the validity of the assay. Serum withdrawal can induce apoptosis in a variety of cells including HepG2 cells. Consequently, there may have an unacceptable level of caspase activity in the control treatments that confounded the interpretation.

Figure 6. Same comment discussed for figure 5 applies to figure 6 for DAPI staining.

Figure 7. The authors still did not evaluate CYP1A1, CY2C9, CYP3A4 expression at the transcriptional and/or translational level. This is conventionally investigated to verify and further characterize modulation observed at the catalytic level. Without this assessment, it is neither possible to conclude a direct effect on cytochrome P450 activity nor conclude on the mode of action. This is a significant weakness in the manuscript and points to the lack of depth in this investigation.

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